

EchoCLEAN RNA CleanUp Kit – Protocol

for single-step removal of inhibitors and impurities from RNA solutions.

This protocol has been developed to eliminate impurities like inhibitors, salts, nucleotides, TRIzol™, phenol, chloroform and other organic solvents, from RNA solutions.

Materials and equipment needed

- 80 to 110 µl of RNA sample, for RNA samples less than 80 µl, dilute with RNase-free water or Tris buffer to a minimum of 80 µl.
- Microcentrifuge with rotor for 1.5 and 2 ml reaction tubes
Important: Switch centrifuge to *relative centrifugal force, rcf* (x g*); if this is not possible please use formula below* to calculate the conversion of round per minute (rpm) into rcf.
- Vortexer
- One reaction tube (2 ml) per sample for column preparation
- One reaction tube (1.5 ml) per sample for elution and collection of the purified RNA
- Pipets for 10 µl and 200 µl scale, corresponding pipet tips
- For fastest procedure (PROTOCOL 1): Cap Puncher (BioEcho product no. 050-001-001)

Preparation before starting

- Set the microcentrifuge to **1000 x g***
- **Important:** *Switch to relative centrifugal force, rcf* (x g*, not rpm)

Product use limitation

The EchoCLEAN RNA CleanUp Kits are for research use only. They have not been registered or authorized to be used for diagnosis, prevention or treatment of a disease.

* Most centrifuges offer the choice between rpm and g-force (rcf); if not, calculate the rpm matching the g-force using the formula: $\text{rpm} = 1,000 \times \sqrt{\frac{g}{1.12 \times r}}$, where r = radius of rotor in mm. and g the required g-force.
E. g., with a radius of 150 mm, the corresponding rpm to 1,000 x g is approx. 2,400 rpm.



BioEcho Cap Puncher

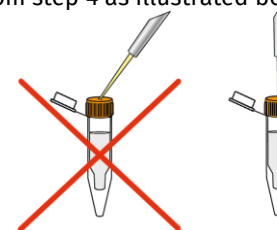
PROTOCOL 1: Purification using the Cap Puncher

Column preparation

1. Vortex the **EchoCLEAN RNA Spin Column** briefly and place into a **2 ml** reaction tube. Let stand for about 5 min.
2. Use of the Cap Puncher (scan QR code to watch a video): Punch a hole into the column cap and lift the column together with the Cap Puncher out of the 2 ml collection tube. Snap off bottom closure of the column and detach the Cap Puncher by twisting while pulling out.
Place the punched spin column back into the 2 ml reaction tube
3. Centrifuge for **1 min at 1000 x g***. Discard the flow-through volume (“void volume”) collected in the 2 ml reaction tube.
4. Place the prepared spin column into a new **1.5 ml** reaction tube for elution of the purified RNA and place back into the rack.
Continue with “Clean up of RNA”.

Clean up of RNA

5. Transfer **80 – 110 µl of the RNA sample** to the prepared **EchoCLEAN Spin Column** from step 4 as illustrated below:



Insert pipet tip vertically through the hole in the column cap and pipet the sample slowly (~5 sec) into the column.

Note:

- Do not punch pipette tip into the resin bed during loading of lysate!
6. Centrifuge **1 min at 1000 x g***. The purified RNA elutes into the 1.5 ml elution tube and can be immediately applied in downstream applications.

The eluted RNA can be used immediately or stored at 4°C or for long-term storage at -80°C. For spectrophotometric analysis, use 1x Tris Buffer (τ) as blank supplied with the kit.

EchoCLEAN RNA CleanUp Kit

for single-step removal of inhibitors, TRIzol™, organic solvents and a wide range of impurities from RNA solutions

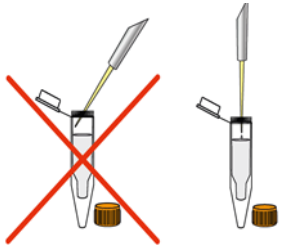
PROTOCOL 2: Purification **without** the use of a Cap Puncher

Column preparation

1. Vortex the **EchoCLEAN RNA Spin Column** briefly and place into a **2 ml** reaction tube. Let stand for 5 min.
2. **Loosen** the screw cap of the spin column **half a turn** and **snap off the bottom closure**.
Important: Do not close the screw cap of the spin column. The screw cap must stay loosened **half a turn** to avoid generation of a vacuum. Place the column back into the 2 ml collection tube and both into the centrifuge.
3. Centrifuge for **1 min at 1000 x g***. Discard the flow-through volume (“void volume”) collected in the 2 ml reaction tube.
4. Place the prepared spin column into a new **1.5 ml** reaction tube for elution of the sample RNA and place back into the rack. Continue with “Clean up of RNA”.

Clean up of RNA

5. Transfer **80 – 110 µl of the RNA sample** to the prepared **EchoCLEAN Spin Column** from step 4 as illustrated below




Open cap and pipet the sample slowly (~5 sec) onto the center of the resin bed of the prepared spin column. Close screw cap and loosen again half a turn.

Important: Do not close the screw cap of the spin column tightly!

Note:

- Do not punch pipette tip into the resin bed during loading of lysate!
6. Centrifuge **1 min at 1000 x g***. The purified RNA elutes into the 1.5 ml elution tube and can be immediately applied in downstream applications

The eluted RNA can be used immediately or stored at 4°C or for long-term storage at -80°C. For spectrophotometric analysis, use 1x Tris Buffer  as blank supplied with the kit.

Product no. (rxn's)	020-002-050-010 (10)	020-002-050-010 (50)	020-002-050-250 (250)
Kit contents	EchoCLEAN RNA CleanUp Spin Columns, 1x Tris Buffer		
Related products	EchoSAFE RNA Gel Loading Buffer 030-003-0005		

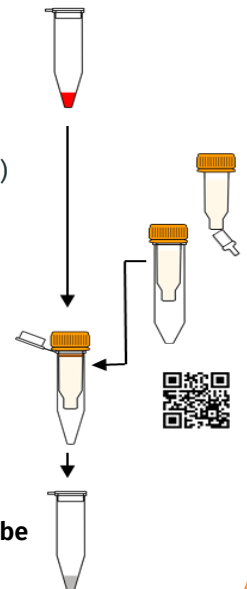
Quick PROTOCOL (please read protocol first)

Column preparation

- Vortex **EchoCLEAN spin column** and place in a **2 ml** tube
Let stand for 10 min
- Punch a hole in the cap with the Cap Puncher, and break off bottom closure (scan QR code to watch a video)
- Place spin column back into 2 ml tube
- Centrifuge **1 min at 1000 x g*** to elute column buffer
- Place column in a **1.5 ml** tube

Clean up of RNA

- Transfer **RNA sample (max. 110 µl)** by pipetting **slowly** through cap hole (scan QR code to watch a video)
- Centrifuge **1 min at 1,000 x g*** to elute RNA into **Elution tube**
- Eluted RNA is ready to use



BioECHO Life Sciences GmbH
Nattermannallee 1
50829 Köln (Cologne)/Germany

Phone: +49 (0) 221-99 88 97-0
E-Mail: contact@bioecho.de
www.bioecho.de